NATURAL PHENOLIC COMPOUNDS IN LEACHATES AND SOIL SOLUTIONS.

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INTRODUCTION

The hypothesis of allelopathic interactions in *Picea abies* (L.) Karst. altitude stands, between trees, understory vegetation (*Vaccinium myrtillus* L.) and spruce seedlings have been strengthened with identification of biologically active compounds in spruce and bilberry, against spruce seedlings and mycorrhizal fungi (Boufalis and Pellissier 1993, Gallet 1994). Bilberry leaves contained high amounts of phenolic monomers (especially caffeic acid and catechol) and flavonoids, while condensed and hydrolysable tannins were abundant in both leaves and roots. Spruce needles were characterized by condensed tannins and especially p-hydroxyacetophenone (i.e. aglycon of picein), a specific and highly variable compound. Most of these compounds have been found in water extracts of litter and associated humus layers of these two species (Gallet and Lebreton, 1995) while their occurrence and concentration in natural solutions is not known. The objective of this study is to determine the phenolic composition (almost phenolic acids *sensu lato*) of the natural solutions in the vicinity of spruce seedlings. These solutions included throughfalls and snow, which could reach the target plant through the aerial parts, and soil solutions which are in close contact with the root system. Moreover, the knowledge of quantitative evolution of the different molecules in the soil profile is essential to estimate degradation rates and persistances of these molecules in the humus layer, which are key factors for expression of the allelopathic potential (Dao, 1987).

MATERIALS AND METHODS

Sampling

Sampling was made at 1860 m in a bilberry-spruce forest (530 trees ha^{-1}) in Tarentaise (Northern-Alps/Savoy/France) on a humo-ferric podzol, characterized by poor regeneration on decaying stumps (Bernier *et al.*, 1993). The understorey was dominated by crowded bilberry areas, with litterfalls of 360 kg ha^{-1} yr^{-1}. The mixed litter (3 cm thick) was made of spruce needles (litterfalls = 650 kg ha^{-1} yr^{-1}), bilberry leaves and moss fragments on a mor humus (pH =3.6, C-to-N ratio = 28.4).

Leachates

Throughfalls were collected during summer 1991 with PVC gutters under three different adult trees, and with micro PE gutters connected to PE bottles under bilberry heath. The samples were collected as soon as possible after rain, and immediatly analysed for phenolic content.

Snow samples were taken during the spring 1991 in PE bags, and analysed immediatly.

Extraction of soil solutions

Soil samples were collected during summer 1993 after rain (once drainage was finished) with an auger and bulked into a single sample for each horizon. Since a minimal volume of 300 ml was necessary for phenolic monomer extraction, the fresh

weight of soil have been estimated to be about 1 kg for A horizon, about 2 kg for E and B. Then, splitt of layers was not possible. After storage at -18°C, they were centrifuged at 5000 rpm (SORVALL RC apparatus, HS4 rotor and swinging buckets) at 4 °C for 90 min, according to Keller (1995). Centrifugal solution was withdrawn from the collection cup with a pipette and put together till 300 ml.

Chemical analysis

Total phenols were determined with FOLIN-CIOCALTEU reagent using gallic acid as a standard (Marigo 1973). Tanning capacity was determined by the colorimetric method of Bate-Smith, modified by Schultz *et al.* (1981), with tannic acid as the standard. Monomeric compounds (phenolic acids *sensu lato*) were extracted from filtered and acidified solutions with ethyl-ether and redissolved in ethanol before HPLC analysis as described earlier (Gallet 1994).

Statistical analysis

Matched data were compared using Wilcoxon signed-ranks test for dependant variables, and mean comparison were performed using U-Mann Whitney test.

RESULTS

Throughfalls

	pH	Total phenols mg l^{-1}	Tanning capacity mg l^{-1}	Phenolic monomers $\mu g\ l^{-1}$	
				pha	others
Control (incident rain)					
13 september	5.6	0.3	0	0	0
25 september	4.9	0.9	0	0	0
2 october	5.3	0.4	0	0	0
Mean	5.3 (0.4)	0.5 (0.3)	0	0	0
Vaccinium myrtillus					
13 september	6.4	4.6	0	0	0
25 september	6.0	2.6	tr	0	caf
2 october	6.4	1.8	0	0	0
Mean	6.3 (0.2)	3.0 (1.4)	-	0	-
*Spruce**					
13 september	-	-	-	-	-
25 september	4.2 (0.2)	27.5 (5.5)	713 (40)	184 (93)	pro, phb : tr
2 october	4.4 (0.3)	22.0 (2.1)	443 (343)	31 (12)	pro, phb : tr
Mean	4.3 (0.2)	24.7 (4.8)	578 (264)	108 (102)	-

Table 1. pH and phenolic composition of incident rain (sampled in a clearing), and of spruce and bilberry througfalls. * Mean (and SD) of three samples. pha : p-hydroxyacetophenone ; caf : caffeic acid ; pro : protocatechuic acid ; phb : p-hydroxybenzoic acid. tr : traces ; - : not determined.

Flowing of incident rain on foliage greatly modified its chemical composition, especially under spruce (Table 1). Under bilberry, pH increased by + 1 and total phenols amount increased by 6 times. Though they are abundant in bilberry leaves, tannins and phenolic acids don't seem to be leached out into the leachates, except some traces (25 september). The chemical modifications in throughfalls on contact with spruce needles and bark are greatly visible as the spruce leachates are highly coloured (brown-orange). Further, pH of the spruce leachates was significantly more acidic (i.e. 4.3) compared to incident rain and the phenolic content was increased by 50 times. Moreover the spruce leachates exhibited high tanning capacity and significant amount of p-hydroxyacetophenone (i.e. 10^{-6} M.).

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The high differences in phenols amounts observed for the same sampling area are due to temporal variations related to rain periodicity and intensity.

Snow samples

	pH	Total phenols mg l^{-1}	Tanning capacity mg l^{-1}	pha µg l^{-1}
Control				
10-20 cm	5.6	0.68	0	0
0-10 cm	5.4	1.42	0	0
*Spruce**				
10-15 cm	5.6 (0.2)	2.30 (1.43)	0	32
5-10 cm	5.5 (0.1)	1.01 (0.32)	0	7
0-5 cm	5.4 (0.1)	3.33 (2.51)	0	tr

Table 2. Phenolic composition of snow sampled on the ground floor of a clearing site and of the spruce forest. * mean (and SD) of three samples

The slight increase in total phenols in snow samples under spruce trees compared to the control site (clearing) was related in part to the occurrence of p-hydroxyacetophenone in the snow (Table 2). No other compounds could be detected.

Capillary waters

	pH	Total phenols mg l^{-1}	Phe	Bal	Pro	Phb	Van	Cin	Pha	Sum
							Phenolic monomers µg l^{-1}			
AO layer										
5 july	3.5	39.8	40.7	3.7	49.3	75.9	164.6	25.2	19.0	378.4
13 july	3.9	29.6	9.9	2.4	9.2	23.5	50.4	27.0	2.7	125.2
13 october	3.5	21.7	0	1.2	18.5	24.8	50.4	45.0	12.2	152.2
Mean	3.6	30.4	16.9	2.4	25.7	41.4	88.5	32.4	11.3	218.6
	(0.2)	(9.1)	(21.2)	(1.2)	(21.0)	(29.9)	(66.0)	(11.0)	(8.2)	(139.1)
E layer										
5 july	3.6	65.9	12.1	6.1	75.5	78.7	692.2	84.6	23.1	972.2
13 july	3.4	18.1	0	0	10.8	22.1	31.9	16.2	4.1	85.1
13 october	3.7	16.4	0	0	6.2	15.2	37.0	14.4	4.1	76.8
Mean	3.6	33.5	4.0	2.0	30.8	38.6	253.7	38.4	10.4	378.0
	(0.2)	(28.1)	(7,0)	(3.5)	(38.8)	(34.8)	(379.7)	(40.0)	(11.0)	(514.6)
B layer										
5 july	4	30.9	0	0	24.6	59.3	383.0	37.8	46.2	551.1
13 july	3.9	9.9	0	0	7.7	12.4	33.6	14.4	1.4	69.5
13 october	4.3	10.0	0	0	9.2	5.5	15.1	10.8	6.8	16.4
Mean	4.1	16.9	0	0	13.8	25.8	143.9	21.0	18.1	222.7
	(0.2)	(12,1)			(9.4)	(29.3)	(207.3)	(14.7)	(24.5)	(284.6)

Table 3. Phenolic composition of the centrifuge waters from the three layers of a podzolic soil (AO : 8-0 cm ; E layer : 0-25 cm ; B layer : 25-50 cm). Phe : catechol + hydroquinone ; Bal : p-hydroxybenzaldehyde ; Pro : protocatechuic acid ; Phb : p-hydroxybenzoic acid ; Van : vanillic acid ; Cin : cinnamic acids (caffeic + p-coumaric + ferulic acids) ; Pha : p-hydroxyacetophenone. Sum : sum of all the identified phenolic acids.

The phenolic analysis of soil solutions of three layers of the podzolic soil (Table 3) revealed only minor differences in quantity and quality of phenolic compounds between the solutions obtained from the AO and the E layer, while more visible variation occured when reaching the B layer. The pH increased, and total phenols amount was reduced to half compared to E layer. Participation of phenolic acids to the total phenolic fraction was low and quite constant with depth : Sum-to-Total phenols ratio (x 100) = 0.7 - 1.1 - 1.3 respectivly in A - E - B layers.

The pattern of the phenolic acids identified in the soil solutions was quite equal for the three layers : vanillic acid > p-hydroxybenzoic acid ~ protocatechuic acid ~ ferulic + p-coumaric acids > p-hydroxyacetophenone > catechol + hydroquinone > p-hydroxybenzaldehyde. Very slight decrease (compared to E) or no variation (compared to A) was observed in B layer solution for the sum of these phenolic acids, while distribution of the monomers was different. The proportion of simple phenols and aldehydes with respect to total phenolic acids (in %) decreased with depth, and they became negligible in E and B layers. The same trend was observed for protocatechuic acid (12%-8%-6% respectivly in A - E -B layers), and cinnamic acids (15%-10%-9%). At the opposite, amounts of p-hydroxyacetophenone (5%-3%-8%) was more stable, and vanillic acid lend to accumulate : 40%-67%-65%. Temporal variations were high, as can be seen for the first sample (5 july) which exhibited greater concentrations of total phenols and of phenolic acids. Though they were certainly related to climatic variations and plant phenology, they were difficult to explain due to few replicates.

DISCUSSION

The phenolic patterns of the natural solutions sampled in the bilberry-spruce stand appear to have a close relation to the vegetation. Throughfalls composition reflects the phenolic metabolism of each species. The ubiquitous occurrence of p-hydroxyacetophenone (snow, leachates and soil) indicates its abundance and its stability in the forest system. According to our knowledge, this is the first report of occurrence in snow of such allelochemicals, which might have be leached from the decaying needles visible in the snow. Its greater concentration near the interface between snow and soil (0-5 cm) compared to the upper parts could be through capillarity from the humus solutions. The extracellular persistance of this compound is probably of great significance in spruce ecology, due to its fungistatic nature (Oswald et al., 1987) and as stress compound involved in spruce die-back (Hoque, 1985). The great input of tannins by spruce leachates in the vicinity of the trunk is of considerable ecological significance as they directly relate to the patterning of vegetation and microorganisms in this area. Both needles and stem bark probably contributed to the release of these tanning components.

In contrast to spruce, the throughfalls of bilberry were less in phenolic content. Caffeic acid, the major phenolic compounds of the bilberry leaves (with 6.3 mg g^{-1} of dry weight) (Gallet and Lebreton, 1995) was identified. pH was higher than the incident rain probably due to greater levels of cations and other minerals (Parker, 1983). The detection of low levels of tannins in bilberry leachates indicates the inability of the leaves to release appreciable quantities of these compounds into leachates, when compared to spruce. Probably the incident rain had a longer exposure and a larger surface of contact with spruce stems and needles, while the crossing of bilberry cover is far quicker.

The important observation made in this study through the podzolic profile is the decrease of total phenols between the surface layers (AO and E) and B layer ; though the sum of total phenolic monomers is quite stable. On the other hand, the variation in the pattern give some indication of the behavior of each compound. For example, protocatechuic acid is known to be a major precursor for ring cleavage (Barz and Weltring, 1985), and its level depends on the activity of microorganisms able to open this aromatic ring. Incorporation in oligomers and polymers, favoured by enzymes and by biotic processes, could equally explained the disappearance of such compounds (as catechol). For other molecules like cinnamic acids, decrease of their levels with depth could be related to their (bio) transformation into benzoic acids. Occurrence and prevalence of vanillic acid is probably due to their continuous liberation, during lignin (« softwood ») degradation. The stability or slight increase of p-hydroxyacetophenone amounts through the profile may be due to low biodegradation process, for lack of specific microorganisms.

The allelopathic effect of p-hydroxyacetophenone on germination and primary growth is well established, owing to bioassays tests performed at 10^{-3} M. or 10^{-4} M (Gallet 1994). But lower concentrations of the natural solutions (about 10^{-6} M. in leachates, 10^{-7} M. in snow and soil solutions) have been shown to be active against mycorrhizal fungi respiration (Boufalis and Pellissier, 1994). The same trend could be noticed for others plant phenolic acids, described by numerous authors as allelochemicals, despite their weak quantities in the soil. Difficulties in quantifying forest soil phenolic compounds had often been underlined, due to spatial heterogeneity and to methodological constraints. No satisfactory methods for capillary waters extraction exists, even centrifugation remains the most desirable technics to collect natural soil solutions (Giesler and Lundström, 1993). Moreover, phenolic monomers are only a minor fraction of the phenolic fraction of forest soil solutions, only approached by « total phenols » determinations. Oligomers and soluble polymers (i.e. fulvic acids) were prevalent in soil solutions, but knowledge of their activity towards plant growth is still in question (Dell'Agnola and Nardi, 1987).

Further, the variability in the levels of these compounds can be related to climatic variations, plant phenology and (micro)biological activity. Thus, the knowledge of conditions which favoured concentrations of capillary waters, for instance during dry periods, or after autumnal rains when leachates carried a lot of compounds from the decaying leaves and litters, is of considerable importance in assessing the allelopathic potential of such compounds.

Under field conditions, the aerial parts of the seedlings would be exposed from time to time to great amounts of p-hydroxyacetophenone and tannins, while the root system will be in permanent contact with at least ten phenolic monomers present in the microporous waters of the organic layer. Though, molecules and biological processes vary among the plant part, synergistic and/or additive effects of these mixtures deserve more attentions understanding complex interactions occurring in forest floor.

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